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Editorial

Taking a Bite out of Malaria: Controlled Human Malaria Infection by Needle and Syringe

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In this issue of *AJTMH*, Roestenberg and colleagues report that healthy adults can be infected by the intradermal injection of aseptic, purified, vialled, cryopreserved *Plasmodium falciparum* sporozoites (PfSPZ Challenge).¹ Because of the potential of this “challenge in a bottle” to standardize and dramatically expand the use of controlled human malaria infections (CHMI) for assessment of malaria vaccines, drugs, and diagnostics, and naturally acquired immunity and innate resistance to malaria, this approach to CHMI may well turn out to be one of the major achievements in malaria vaccine research and development of the past half-century.

CHMI by the bites of five infected mosquitoes has become widely accepted as a safe^{2–4} and informative initial step in evaluating the efficacy of pre-erythrocytic stage vaccines.^{5–7} It has also played a significant role in the development of anti-malarial drugs^{8–10} and diagnostic assays¹¹ and in the assessment of host immune responses to infection.^{12,13} At last count in the published literature, over 1,300 clinical trial subjects had been exposed to CHMI^{5,6} since CHMI using mosquitoes that had fed on *in vitro* cultures of *P. falciparum* was introduced in 1986.¹⁴ Investigators from multiple international centers, together with representatives from the World Health Organization (WHO), recently generated a standardized document for the design and conduct of CHMI and a second document for the microscopy methods used to determine the patency endpoint.¹⁵

Nevertheless, CHMI based upon the bites of five infected mosquitoes has had limitations, which can now be addressed by being able to use “vialled” sporozoites administered by needle and syringe for CHMI. The most important limitation has been that CHMI has been restricted to only a few centers worldwide, because of the requirement for having *P. falciparum*-infected mosquitoes. Now that researchers at Sanaria have developed the methods for manufacturing, characterizing, and shipping aseptic, purified, cryopreserved PfSPZ that meet all regulatory standards, CHMI will be available to investigators at any clinical research center in the world, including malaria-endemic countries.

Another limitation has been the variability in the time from mosquito bite inoculation to the first detection of sporozoites in the blood stream (pre-patent period) between centers, clinical trials conducted at the same center, and volunteers inoculated on the same day in the same clinical trial at the same center. This has probably been a result of the fact that infectivity of sporozoites, the number of sporozoites per mosquito, and the numbers of sporozoites inoculated by the bites of five infected mosquitoes varies from center to center, between trials at the same center, and even between individuals in the same trial at the same center. Using vialled sporozoites that have met defined lot release specifications should

reduce or eliminate these variables, as a defined number of sporozoites with the same quality control release specifications can be used for all comparative studies. In trials of interventions this will allow investigators at different sites to not only compare infectivity rates, but also to reliably compare pre-patent periods after specific interventions.

It is uncommon in the field for any individual to be exposed to the bites of five *P. falciparum*-infected mosquitoes at the same time, or even on the same night. This has led some investigators to argue that exposure to five infected mosquitoes in CHMI does not adequately reflect what occurs in the field. Using injected sporozoites, it should be possible to define how many sporozoites will infect 50%, 75%, or 90% volunteers and potentially use those numbers, instead of the 100% infectious dose. Furthermore, more than 95% of all CHMIs since 1986 have been done with the NF54 strain of *P. falciparum* or one of the clones (3D7 and CVD1) derived from this strain. CHMI with vialled sporozoites should allow for additional isolates that differ significantly at the genomic level to be used.

On a different note, PfSPZ Challenge could, itself, become a component of a vaccine. The same authors from Radboud University Nijmegen Medical Center (RUNMC) recently showed that high-level sustained protective immunity to *P. falciparum* infection (up to 28 months) could be induced through immunization by the bites of infected mosquitoes under cover of chloroquine chemoprophylaxis.^{4,16} Clinical trial testing of this model, with exposure to infected mosquito bites replaced by PfSPZ Challenge, is in progress.

Finally, the research being conducted on PfSPZ Challenge is central to the development of a whole organism malaria vaccine, regardless of whether the parasite is attenuated by radiation, genetic modification, or concurrent chemoprophylaxis. The whole organism approach is believed by many to be the only strategy that can generate high-level, sustained sterile protection against infection and that can be used for eradication. It has now been over 40 years since the first clinical trials demonstrated complete protection generated by exposure to mosquitoes carrying irradiated sporozoites.^{17–19} It was recently shown that humans could be immunized safely with the PfSPZ Vaccine (aseptic, radiation-attenuated, purified, vialled, cryopreserved *P. falciparum* sporozoites) with some level of (albeit suboptimal) immunogenicity and efficacy.²⁰ The current publication by Roestenberg and others provides data that conclusively demonstrates that the technology upon which this approach is built **works**. Aseptic, purified, vialled, cryopreserved sporozoites can reach and infect liver cells. Furthermore, in the case of PfSPZ Challenge, they can develop in hepatocytes and erythrocytes to normally functioning merozoites.

Nevertheless, this study by Roestenberg and colleagues is just a first step. The next hurdle will be to optimize the administration of PfSPZ Challenge and demonstrate that healthy adult subjects can be consistently and reliably infected by the parenteral route, taking the result of 5/6 subjects infected

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per group to 100% infectivity with a pre-patent period similar to that achieved with exposure to five infected mosquitoes. Animal models indicate that the optimal route for both PfSPZ Challenge and the PfSPZ Vaccine is intravenous administration.²⁰ A dose-escalation trial of PfSPZ Challenge administered IV is now in progress and results are eagerly awaited; an IV trial of the PfSPZ Vaccine is also in progress. If IV PfSPZ Challenge is shown to safely and reproducibly infect subjects, it will be another giant step forward. Meanwhile, investigators are continuing to try to optimize non-IV routes and methods of administration, including microneedle array. Murine studies indicate that multiple injections with smaller volumes may be optimal.²¹ Once optimal IV and non-IV regimens are established, it will be important to compare IV and non-IV administration of PfSPZ Challenge with mosquito bite administration in CHMI studies, particularly for vaccines designed to induce antibodies against sporozoites.

We are clearly entering a new and exciting era in which CHMI using injected sporozoites will be able to be used at clinical centers worldwide to rapidly and efficiently assess new malaria vaccines, drugs, and diagnostics, and naturally acquired immunity and innate resistance to malaria.

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